ON THE UPTAKE OF CARBON DIOXIDE AND BICARBONATE BY ROOTS, AND ITS INFLUENCE ON GROWTH[1]

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From the viewpoint of the efficient use of light in photosynthesis, algae possess the advantage (over higher plants) that the medium in which they grow can be readily enriched with bicarbonate, and growth correspondingly increased. For higher plants, unfortunately, it would hardly be possible to enrich the atmosphere appreciably with CO_2. However, it occurred to one of us that it might be practical to enrich the soil or the nutrient solution (17). One or two unsuccesful attempts at this have been made in the past, but without any systematic study of the factors involved.[4] Recent claims of increased yields following carbonate fertilization, by Kursanov and coworkers in Russia (see below) make a study of this problem more urgent. Such a practice would depend critically on whether roots can absorb CO_2 or bicarbonate to an appreciable extent. The present work was therefore undertaken to determine the amount of CO_2 or bicarbonate which could be absorbed by the roots of intact plants, and to study the effect of CO_2, applied in the root medium, on the growth of the root system and of the whole plant. Both monocotyledons and dictyledons have been included in the experiments.

It is known, of course, that roots, like other non-green tissues, are capable of fixing CO_2. Ruben and Kamen (14) demonstrated uptake of $C^{11}O_2$ by a preparation of ground barley roots as long ago as 1940, but the short half-life of the isotope used prevented identification of the compounds into which the CO_2 was incorporated. Overstreet, Ruben and Broyer studied the uptake of bicarbonate ions by excised barley roots over a short period (11). Their data allow an approximate comparison of the amounts taken up with the amount produced by respiration; with their "low-salt" plants, using $KHC^{13}O_3$ as bicarbonate source, it develops that the uptake represented about 10 % of respiration. However, the amount of K^+ taken up was three to six times as large as the amount of HCO_3^- taken up in the same length of time. More recently, Poel (12) has repeated and extended these experiments, using C^{14} and radiochromatographic techniques, and has identified the products of fixation as malic, citric, aspartic and glutamic acids, serine, asparagine, glutamine and tyrosine, with a very little α-keto-glutaric acid.

Kursanov, Kuzin and Mamul (6) studied the uptake of $C^{14}O_2$ by the roots of intact bean seedlings, and found that after an 18-hour exposure in the light most of the radio-activity was in the stems, indicating that the fixation products had been translocated upwards. In a later paper, Kursanov, Krjukova and Vartapjetjan (5) describe more detailed experiments in which they were able to demonstrate the fixation products of CO_2-uptake in the shoots after only 15 minutes exposure. These authors estimate that under their experimental conditions the amount of carbon dioxide absorbed by the roots is as much as 25 % of that taken up from the atmosphere by the leaves. Kursanov (4) reported later that soluble carbonates introduced into the soil together with fertilizers increased the yield of several crops by up to 18 %, and Grinfel'd (1) states that 30 or 50 Kg CO_2 per hectare, supplied as ammonium carbonate, increased the yield of sugar beets 7 and 16 % in two trials, although in the first part of the season the growth seems to have been decreased by the treatment. The Russian investigations thus appear to indicate that the uptake of CO_2 by roots is considerable and has a beneficial effect on growth.

Most of the work mentioned at the beginning on the effect of carbon dioxide and bicarbonate has been done with excised root systems, in which the translocation described by Kursanov and coworkers could not, of course, be observed. However, there is some evidence that under certain conditions an excess of bicarbonate and of carbon dioxide in the root medium may have a detrimental effect on growth, through a condition known as lime-induced chlorosis (cf experiments in (13)). Some workers have concluded that part of the deleterious effect of alkaline soils is due to the bicarbonate ion, although in general it is carbonate rather than bicarbonate which appears to exert toxic effects on roots (9). Hassan and Overstreet (2) in a study mainly seeking to relate the deleterious effects of alkali soils to the influence of sodium and other cations, did note that the growth of seedling radish roots was inhibited much more by $NaHCO_3$ than by NaCl.

As against evidence of growth-inhibition, Hoagland and Broyer, in their studies of salt uptake by excised barley roots, did not find any effect of carbon dioxide, up to 10 % in air, on the rate of salt uptake. A combination of 20 % CO_2 and 10^{-3} M HCO_3^- had no effect on the uptake of K^+ (3). Under the same conditions there was a 15 % decrease in bromide accumulation, but the authors do not consider this very significant. Contrasted with this, Steward and Preston (16), studying potato discs, found that at pH 5.5 a concentration of about 20 millimoles of bicarbonate can inhibit the bromide uptake of potato discs completely.

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[4] In particular, experiments by I. Spear and K. V. Thimann, in 1953, demonsrated that pea plants could not be grown successfully when the sole supply of CO_2 was through the root.

It is clear, therefore, that the data so far available on CO_2 uptake by the roots, and its effects on the plant, are scanty, and some of them point in opposite directions. For this reason a further study seemed well justified.

Methods

Seeds were germinated on wet filter paper and the seedlings were transplanted into gravel which had been washed several times with dilute HCl. The cultures were sub-irrigated once a day with the nutrient solution given in table I.

Table I

Composition of the Nutrient Solution

Major elements millimoles/l		Micronutrients, mg/l	
KNO_3	10	$FeSO_4 \cdot 7\ H_2O$	5
$CaSO_4$	4	$MnCl_2 \cdot H_2O$	1
$MgSO_4$	2	$Na_2B_4O_7 \cdot 10\ H_2O$	20
$Ca(H_2PO_4)_2$	1	$CuSO_4 \cdot H_2O$	0.1
$(NH_4)_2SO_4$	1	$ZnSO_4 \cdot 7\ H_2O$	0.2

The pH of the nutrient solution was 5.90. After sub-irrigation the root medium consisted of gravel : nutrient solution : air, in the proportions of 9 : 1 : 5. The control pots were flushed with air from which CO_2 had been removed, and the experimental pots with air containing various percentages of CO_2, both at the rate of 5 liters per hour per pot. It was established that the air enriched with CO_2 caused only small changes in the pH of the nutrient solution; in all cases the change was less than 0.2 pH unit. Air was obtained from a compressed air line, the CO_2 being removed from it by bubbling through 20 % KOH. Air with various percentages of CO_2 was obtained by continuous mixing with high purity CO_2. All experiments were done in a greenhouse kept at approximately 25° C.

For determination of the uptake and fixation products $C^{14}O_2$ and $HC^{14}O_3^-$ were used. Roots of intact plants were submerged in a solution containing 1 microcurie of C^{14} per ml. This solution was prepared by diluting a stock solution containing 69 mg of $Na_2C^{14}O_3$ per ml, at an activity of 0.108 millicurie per ml, with aerated tap water and then bringing the pH to 7.5 with 0.01 *N* HCl. The specific activity of the CO_2 in the final solution was 3.1 microcurie per mg CO_2; the concentration of CO_2 was 0.56 millimolar and that of HCO_3^- ions was 6.58 millimolar. The solution was in equilibrium with a partial CO_2 pressure of 1.65 %.

In treatments in which the plants were exposed to light, incandescent lamps were used and the light intensity was adjusted to about 50,000 ergs/cm^2 × sec between 400 and 700 mμ. The plants used in these experiments were between 10 and 15 days old, and had been grown in vermiculite and tap water in the greenhouse.

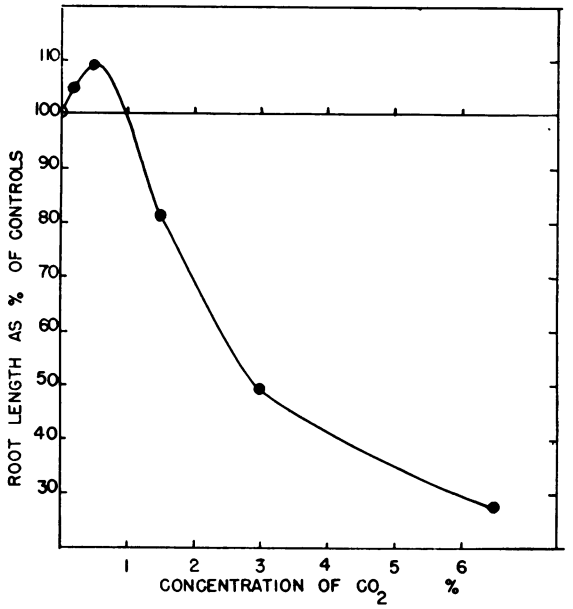

Fig. 1. Final root length of peas (*Pisum sativum* var. Alaska), after a 10- to 13-day growth period, as a function of CO_2 concentration in the root atmosphere. Each point the mean of 20 plants. Inhibition similar to that indicated at 6.5 % CO_2 was shown also by *Vicia Faba*, *Phaseolus vulgaris* and *Helianthus annuus*.

Results

The Effect of CO_2 and HCO_3^- on Growth of the Root System: *Pisum sativum* var. Alaska and *Avena sativa* var. Victory were used in most of these experiments. The results of a typical series of experiments, recorded after a 10- to 15-day growth period, are given in detail in table II and plotted in figures 1 and 2. Under the experimental conditions used, peas show a strong inhibition of root growth at CO_2 percentages greater than 1 %. They also show a small but significant stimulation of root growth at

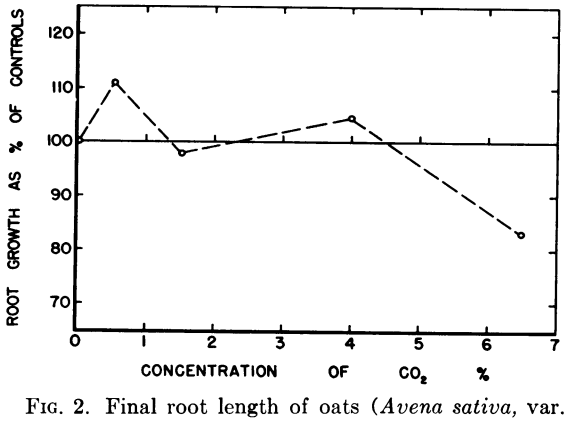

Fig. 2. Final root length of oats (*Avena sativa*, var. Segrehaver) after a 7- to 15-day period, as a function of CO_2 concentration in the root atmosphere. Similar data were obtained with barley.

TABLE II

LENGTHS OF ROOTS OF INTACT PLANTS GROWING IN LIGHT IN NUTRIENT SOLUTION SUPPLIED WITH CO_2-ENRICHED AIR

DURATION DAYS	CO_2 %	ROOT-LENGTH, CM		ACCELERATION (+) OR INHIBITION (−), %
		CONTROL IN CO_2-FREE AIR	WITH ADDED CO_2	
		Peas (Pisum sativum)		
10	0.2	19.9 ± 0.4	21.0 ± 0.3	+ 5.5
10	0.4	19.7 ± 0.6	21.2 ± 0.5	+ 7.6
11	1.5	21.8 ± 0.5	17.6 ± 0.5	−19.2
10	3.0	14.5 ± 0.3	7.5 ± 0.2	−48.3
13	6.5	21.7 ± 0.5	Died	(−73.0)
		Oats (Avena Sativa)		
12	0.6	18.4 ± 1.1	20.3 ± 2.1	+10
15	1.6	18.5 ± 1.5	18.2 ± 1.8	− 2
10	4.0	13.4 ± 0.4	14.0 ± 0.3	+ 4
12	6.5	16.7 ± 0.9	12.7 ± 1.25	−24
7	6.5	10.7 ± 0.3	9.7 ± 0.4	−10

CO_2 concentrations around 0.5 %. Oats, on the other hand, show barely significant growth responses, even at CO_2 concentrations as high as 6.5 %.

Since the curve obtained for peas shows a certain amount of distortion due to the initial length of the roots, a separate series of experiments was made in order to follow the effect of CO_2 directly on the rate of root growth. Pea seedlings, 15 days old, were planted in gravel in long glass tubes, 1.5 inches in diameter, in light, and the medium was sub-irrigated in the way described above. This method was chosen because the roots have a tendency to grow along the glass wall, making it possible to measure the growth rate of a number of roots over short time-periods. For each CO_2 concentration 4 tubes with 3 plants in each were used, and generally 3 roots measured on each plant. Thus each growth rate is based on about 36 root tips. For each CO_2 concentration a control set of plants with CO_2-free air was grown in parallel. The growth rate of these control roots varied from 0.53 to 0.62 mm per hour, except in one group where it was 0.39 mm per hour. Growth was measured over 24-hour intervals. The results, corrected for the differences in rate of the controls from one series to another, are given in figure 3. The difference between the curves of figures 1 and 3 is due to the fact that figure 1 gives only the final lengths without correction for the lengths at the start, while figure 3 presents the elongation in one 24-hour period. The small growth promotion at 0.5 and 0.7 % CO_2 is again observed. The inhibition due to higher concentrations of CO_2 is seen to be even larger than in figure 1, reaching 80 % at about 2 % CO_2.

Several other species were examined as to their response to a concentration of 6.5 % CO_2 in the root atmosphere. *Phaseolus vulgaris, Vicia Faba* and *Helianthus annuus,* grown in gravel with nutrient solution as above, all showed a complete inhibition of root growth at this concentration. *Hordeum vulgare,* six-row barley, proved to be quite unaffected, however, and thus was similar in its behavior to oats.

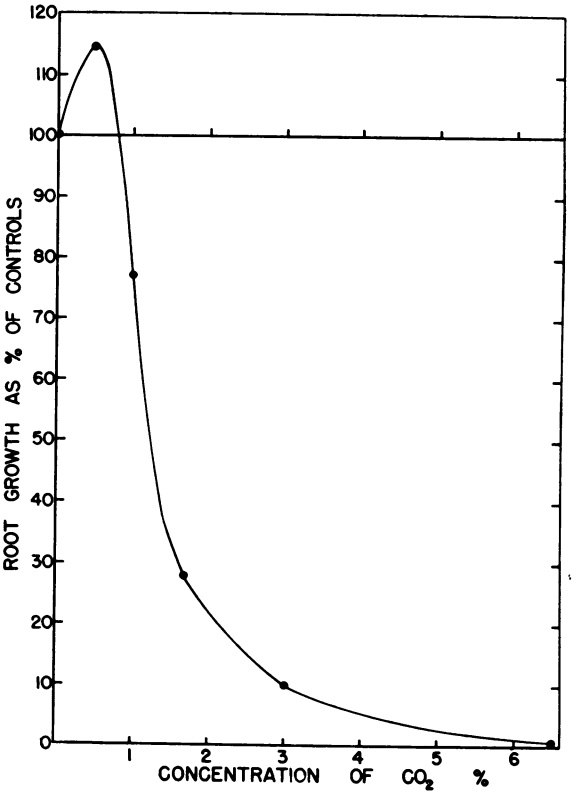

FIG. 3. Increment in root length of Alaska peas, in 24 hours, as a function of CO_2 concentration in the root atmosphere.

Assuming that the solubility coefficient for CO_2 is the same in the solution as it is in water, the Henderson-Hasselbalch equation gives the concentrations of CO_2 and HCO_3^- in the liquid root medium, at pH 5.90, shown in table III.

In the experiments described above, the inhibition of root growth occurs at much lower concentrations of CO_2 and especially of HCO_3^-, than the inhibitions reported by Porter and Thorne (13). However, these authors used a nutrient solution of much higher pH value, viz between 7.3 and 8.5, which is far from optimal for many plants, and at which the percentage of free CO_2 would be small. Similarly, in the data of

TABLE III

CALCULATED CONCENTRATIONS OF CO_2 AND BICARBONATE IN EQUILIBRIUM WITH CO_2-ENRICHED AIR IN A SOLUTION AT PH 5.90

CO_2 IN GAS STREAM, %	CO_2, MILLIMOLES/L OF SOLUTION	HCO_3^-, MILLIMOLES/L OF SOLUTION
1	0.34	0.11
2	0.68	0.22
3	1.02	0.33
4	1.36	0.44
5	1.70	0.55
6.5	2.21	0.72

Hassan and Overstreet (2), concentrations of bicarbonate ten times the highest value in table III gave only about 12 % inhibition of root elongation. It seems clear, therefore, that it is the CO_2, rather than the bicarbonate, which is the effective agent.

The next step was to determine the extent of CO_2 or HCO_3^- uptake by the roots, and to investigate whether the products of fixation or their translocation could account for the difference in response between the two groups of plants.

Uptake of CO_2 and HCO_3^- by Roots; Fixation Products and Their Translocation: The root systems of five 10-day-old barley plants and five 15-day-old pea plants were submerged in 200 ml solution of $NaHC^{14}O_3$, prepared as described under Methods, and containing 1 microcurie per ml. After 24 hours exposure in the light, the roots were rinsed thoroughly with tap water, the cotyledons removed, and both root systems and shoots were killed in boiling 70 % ethanol. The extracts were filtered, and the ethanol evaporated off from the filtrate, at 55° C and under vacuum. The dry residue was extracted with ether acidified with HCl, the ether extract was decanted off and evaporated, and the residues redissolved in 70 % ethanol. Aliquots of the ethanol and ether extracts were then counted in a gas flow counter. The insoluble fibrous residue was dried, ground to a powder, and also counted. The results, after the usual corrections, are given in table IV, together with the dry weights of the fractions. It will be seen that the pea plants take up five to six times as much $C^{14}O_2$ as the barley. The distribution of radio-activity be-

Table IV

Distribution of C^{14} Taken Up by Roots as $C^{14}O_2$ and $HC^{14}O_3^-$, in Barley and Peas, after 24 Hours' Exposure in the Light (All Data per 5 Plants)

Plant	Dry wt, mg	Total uptake μc	Ethanol fraction μc	Ether fraction μc	Insol fraction μc	Total uptake μc/gm dry wt
Pea shoots	117.0	0.38	0.32	0.03	0.03	3.3
Pea roots *	30.1	0.52	0.27	0.22	0.03	17.3
Barley shoots	30.0	0.08	0.04	0.03	0.01	2.7
Barley roots	20.8	0.09	0.03	0.05	0.01	4.3

* Cotyledons removed.

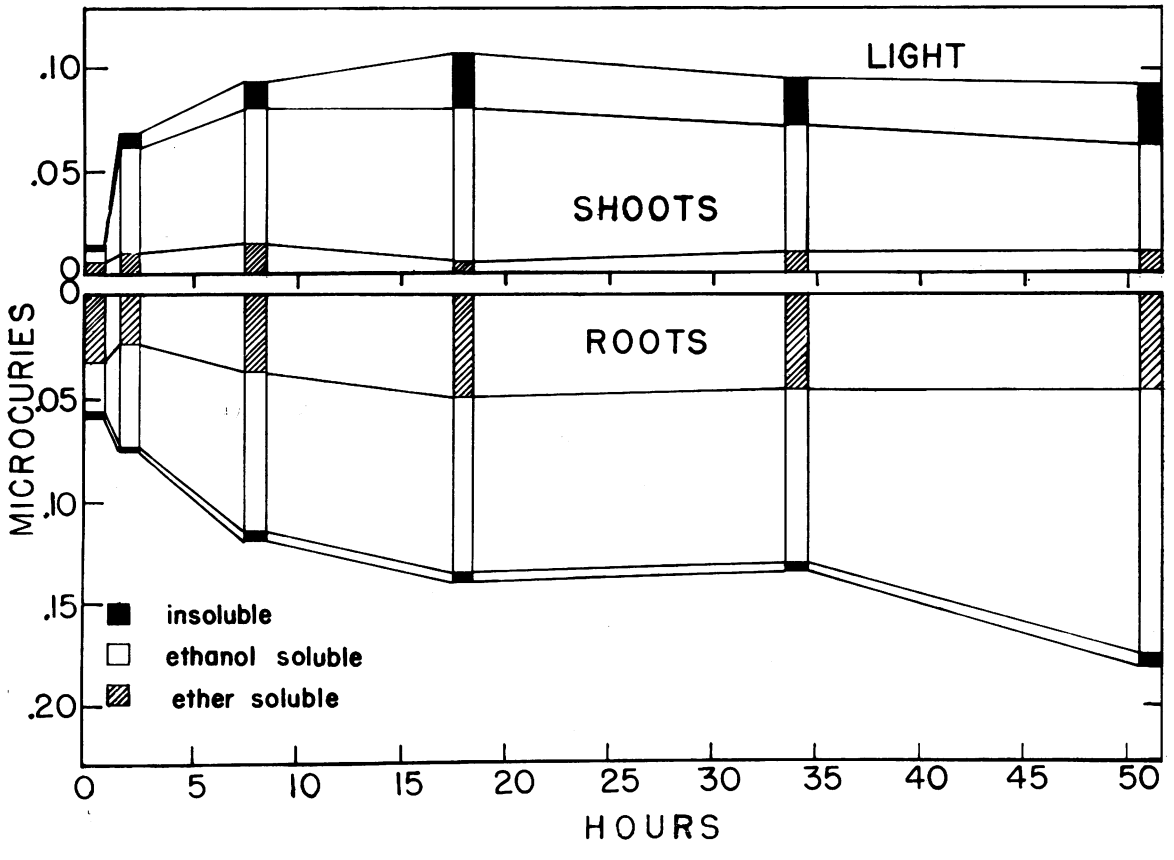

Fig. 4. Time course of uptake and distribution of C^{14} in intact pea plants in light, with the roots exposed to a solution of $C^{14}O_2$ and $HC^{14}O_3^-$.

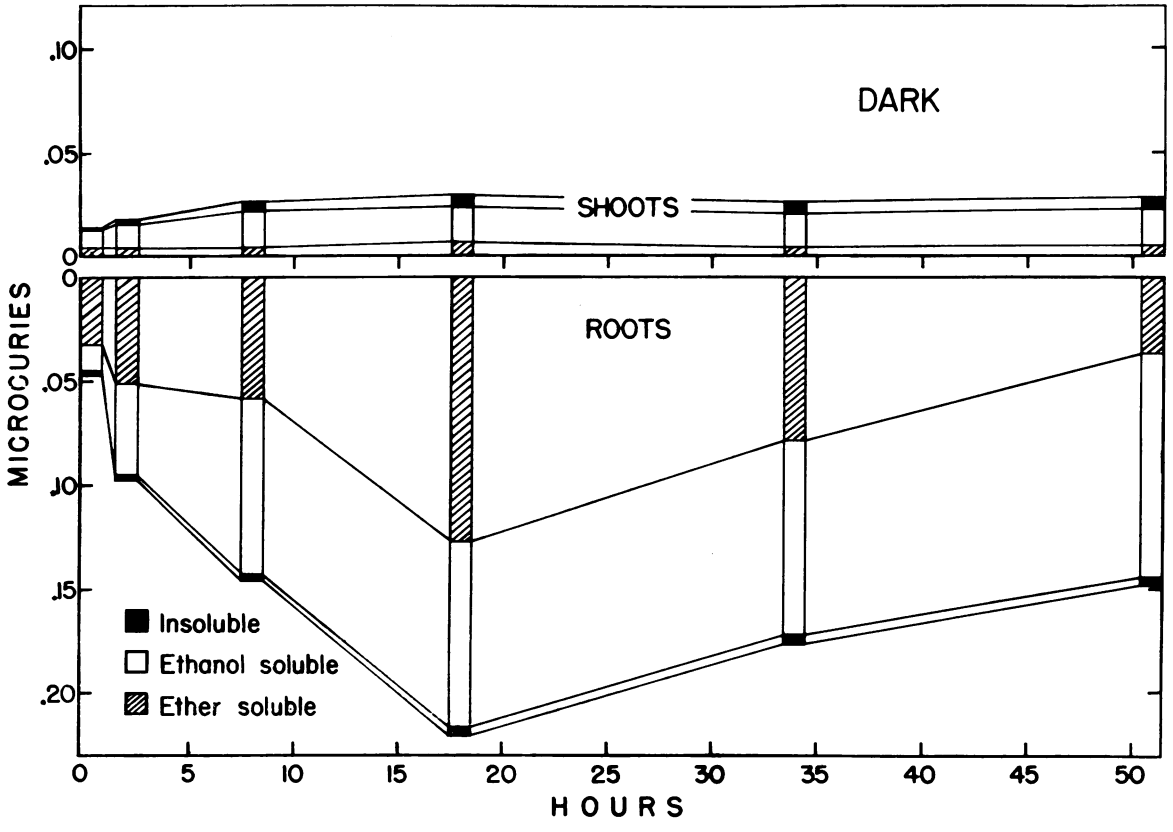

FIG. 5. Time course of uptake and distribution of C^{14} in intact pea plants in darkness, with the roots exposed to a solution of $C^{14}O_2$ and $HC^{14}O_3^-$.

tween roots and shoots is roughly equal, unlike the Russian findings (4). It is also evident that, although the ethanol fraction contains more than half the activity, the ether-soluble material is also quite active. The ether and ethanol fractions were examined by one-dimensional paper chromatography, the distribution of radioactivity on the paper being recorded with a gas flow counter and a recording count-rate meter. It was found that the ether soluble fraction of extracts of both plants had about 60 % of its total activity in malic acid, and 30 % in citric acid, although they appeared to contain a larger absolute amount of citric than of malic acid, as indicated by staining. The ethanol soluble fraction had about 55 % of its activity in sucrose, glucose and fructose, in order of decreasing importance, with the remainder distributed in several spots which were not further identified, but are probably amino acids. The alcohol insoluble fraction was not analyzed. None of the preparations obtained lost any activity as a result of treatment with HCl and subsequent heating to 70° C, indicating that very little, if any, of the absorbed carbon was present in the form of carbonate or bicarbonate.

The time course of the uptake, incorporation and translocation into the shoots, in dark and in light, was determined in peas over a period of 51 hours. The results, based on 5 plants from each determination, are summarized in figures 4 and 5. These data show that the uptake stops after about 8 hours, with 50 % of the ultimate uptake reached in about one hour. There is a considerable difference in the translocation to the shoot, depending on illumination of the shoot; in darkness the amount of translocation is very small. In the thought that the increased translocation might be due to passive uptake with the water

TABLE V

COMPARISON OF THE TRANSPIRATION OF PEA SEEDLINGS IN LIGHT AND DARKNESS WITH THEIR UPTAKE OF C^{14} FROM THE ROOT MEDIUM, AND ITS TRANSLOCATION TO THE SHOOTS

	DARK	LIGHT
Transpiration rate, mg H_2O/hr, 5 plants	215	510
C^{14} absorbed, 15-min exposure		
μC present in vol absorbed	0.05	0.13
μC uptake observed:		
Total	0.06	0.07
Shoots	0.01	0.01
C^{14} absorbed, 2-hr exposure		
μC present in vol absorbed	0.43	1.02
μC uptake observed:		
Total	0.11	0.14
Shoots	0.02	0.07

transpired, the rates of transpiration of comparable pea plants in light and in darkness were determined in a separate experiment. Table V shows the results obtained, with the implications for the uptake in the experiments described above. The data show that in 2 hours of exposure the uptake of CO_2 and HCO_3^- is much smaller than would be expected on the basis of passive absorption with the transpiration stream. Even during the period of most rapid uptake, during the first 15 minutes, the uptake of CO_2 and HCO_3^- shows no sign of selective accumulation. It is evident that the increased translocation in the light parallels the increase in transpiration rate.

In order to compare the uptake of CO_2 and bicarbonate by the roots with the respiratory production of CO_2, respiration rates of isolated root systems were determined manometrically, using plants strictly comparable to those of table V. The data of table V were calculated to CO_2-uptake per hour, and the results compared (table VI).

It will be seen that the uptake of CO_2 in peas is much larger than that in barley, if determined over a 24-hour period, as noted above. If the same comparison is made over shorter absorption periods the difference between barley and peas is smaller, indicating that the barley uptake is saturated in a shorter period of time. The general magnitude of the figures in the last column agrees well with those obtained from Poel's data (12). It should be remarked that the respiration measured is only that of the root system; the respiration of the whole plant is several times higher, and the amount of CO_2 used in photosynthesis is many times higher still, under favorable conditions. Thus it must be concluded that, under the experimental conditions used here, the uptake of CO_2 by the roots must be considerably less than 1 % of the amount of CO_2 taken up by the leaves in photosynthesis.

After the present study was completed, Miller and Evans (10) reported that bicarbonate ions inhibit the activity of cytochrome c oxidase. This finding raised the possibility that the difference between barley and peas in their response to CO_2 and HCO_3^- might be due to a difference in the terminal oxidases of the roots. Since the most characteristic property of cytochrome oxidase is the light-reversible inhibition by CO, a few measurements of the effect of CO in dark and in light on the respiration of excised root systems

TABLE VI

UPTAKE OF C^{14} BY ROOTS COMPARED WITH RESPIRATION RATE OF THE ROOT SYSTEM

PLANT	RESPIRATION	UPTAKE BY THE ROOTS $\mu L\ CO_2/HR$	$\frac{\text{UPTAKE}}{\text{RESP.}} \times 100$
Pea	96	6.0	6.2
Barley	53	1.1	2.1

Both data calculated to microliters CO_2 per 5 plants per hour. Period 24 hours. Solution: $C^{14}O_2 + HC^{14}O_3^-$ as in text.

TABLE VII

LIGHT-REVERSIBLE INHIBITION OF ROOT RESPIRATION BY CO

ROOTS	O_2 CONSUMPTION, $\mu L\ O_2/HR \times GM$ FRESH WT			% INHIBITION	
	AIR	CO/O_2 (9:1)			
		LIGHT	DARK	LIGHT	DARK
Barley	369	300	161	19	56
Pea	303	243	142	20	53

of both plants were made. Whole root systems in moist air were used. In both barley and peas, root respiration was inhibited about 60 % in a gas mixture of 90 % CO and 10 % O_2, while in white light this inhibition was reduced to 20 % (table VII). These results, while not extensive enough for detailed analysis, indicate that in both plants cytochrome c oxidase is at least the predominant oxidase. Apparently, therefore, the inhibition of cytochrome oxidase reported by Miller and Evans does not account for the difference in sensitivity to bicarbonate of these two plants.

DISCUSSION

The experiments described above clearly indicate that relatively low concentrations of CO_2 and HCO_3^- in the root medium strongly inhibit root growth in peas, while less extensive data show the same thing for three other dicotyledenous plants. The same levels of CO_2 and HCO_3^- have no appreciable effect on the two cereals. It might be noted also that the uptake of ions by barley roots (3) appears more resistant than that by potato (16), though the two researches involved are not very comparable. The amounts of carbon dioxide and bicarbonate taken up by representatives of the two types of plants are different (table VI), but the difference hardly seems great enough to account for the complete absence of inhibition in the cereals. Furthermore, there does not seem to be any major difference in the types of fixation products, nor in their translocation into the shoots. A differential effect on the terminal oxidase appears to be ruled out. The small amount of CO_2 taken up must be incorporated into other compounds almost immediately, since none of it is found as carbonate. The effect is not due to formation of bicarbonate, as was suggested by Lindsay and Thorne (7) for the effect of CO_2 in increasing chlorosis, because the concentrations of bicarbonate ion used in the present experiments were less than 1 millimolar. It is thus apparently due to CO_2 itself. It seems, therefore, that some specific toxic effect, to which oats and barley are immune, is exerted by CO_2 in the dicotyledons.

The actual amounts taken up through the roots are in good agreement with estimates derived from the findings of Overstreet et al (11), and of Poel (12). It is not inconceivable that the CO_2 produced in res-

piration arises closer to the fixing sites in the roots than the CO_2 being absorbed from the root medium, with the result that the technique used might yield too low an estimate of the fixation of CO_2. However, the resulting error in the interpretation of the data obtained with $C^{14}O_2$ cannot be very large, since such preferential recirculation of respiratory CO_2 would result in deviations from unity in the respiratory quotient, which are not observed. The absence of appreciable amounts of carbonate or bicarbonate contrasts with the experience of Smith and Cowie (15) with sunflower leaves, in which much of the CO_2 fixed was in reversible combination, which was ascribed to bicarbonate.

The fate of the fixation products, as far as they were determined, seems to be compatible with the pathways suggested by Kursanov (4), but the importance of CO_2 taken up from the root medium appears to be considerably smaller than Kursanov has concluded. Furthermore, Kursanov does not mention the inhibiting effect of relatively low concentrations of CO_2 and HCO_3^- in many species. It should be noted that in both types of plants the uptake ceases after a relatively short time, and the major fixation products are compounds already present in much higher concentrations under normal conditions, so that only a very small increase in their concentration is likely to occur.

In their experiments which led to increased yields of beans, barley and sugarbeet, Kursanov and coworkers (4, 5 and 6) added soluble carbonates in the form of fertilizer, presumably in amounts not exceeding a few hundred pounds per acre. It is interesting to compare this with the normal production of CO_2 by typical soils. Determinations made by Lundegårdh (8) showed that the CO_2 production, in mg per hour per square meter, ranged from 452 in garden soil to 671 in a sandy loam, in a study covering a large number of soil types. Taking an average figure of 600 mg per hour per square meter, this would correspond to about 4000 lbs of CO_2 per acre per month, or well over 10,000 lbs in a growing season. It seems unlikely, therefore, that the increases in yield mentioned by Kursanov could be due specifically to the carbonate added. It is not clear either that the influence of the nitrogen added as ammonium was completely ruled out, especially in Grinfel'd's experiments (1). These facts, taken together, make it improbable, unfortunately, that fertilization with bicarbonate could have much agricultural value.

The concentration of bicarbonate used in the C^{14}-uptake experiments, 6 millimolar, is comparable to the concentrations recorded in soil solutions from agricultural soils, while soil gases may contain from 0.15 to over 2.5 % CO_2 and figures as high as 12 % have been recorded (8, 18). It follows, therefore, that the levels of CO_2 and bicarbonate in the soil must often be high enough to inhibit root growth of dicotyledons, and it may well be that much of the benefit of frequent cultivation of crops derives from the improved diffusion of CO_2 from the soil into the atmosphere. Cereals, on the other hand, judging from their lack of response to high CO_2 concentrations, would appear not to need cultivation for this reason.

Summary

The growth of roots of *Pisum sativum*, *Vicia Faba*, *Phaseolus vulgaris* and *Helianthus annuus* is completely inhibited if the root medium is aerated with 6.5 % CO_2 in air. *Avena sativa* and *Hordeum vulgare* are unaffected by such a treatment. Peas show a small but consistent stimulation of root growth when the root atmosphere contains 0.5 % CO_2, but are clearly inhibited at levels as low as 1.5 %. Some possible explanations of the difference in sensitivity to CO_2 of peas and barley have been ruled out, and a specific toxic effect seems indicated.

The uptake of CO_2 by the roots of both peas and barley is of the order of only a few percent of the amount produced by respiration in short term experiments, and it virtually ceases after about 8 hours. The bulk of CO_2 fixed was converted to malic and citric acids and sugars; after 24 hours only about 10 % had entered the alcohol-insoluble fraction.

Translocation of the products into the shoots of the intact plant was about 3 times as great in the light as in the dark.

It is concluded that carbonate fertilization of crops is unlikely to be beneficial, and that the CO_2 content of some soils may, indeed, already be supra-optimal.

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SULFHYDRYLS IN PLANTS. I. REACTIONS WITH GROWTH REGULATORS[1]

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Many studies have indicated a close connection between sulfhydryl substances and plant growth processes (21). For example, many growth inhibitors are able to combine with sulfhydryls. Several recent studies have led to suggestions of various schemes which involve sulfhydryls in the possible mechanisms of auxin action (8, 12, 17, 21, 23). While there is much interest in sulfhydryls, very little is known concerning their occurrence in plants and their changes during growth (2, 20).

The present work was undertaken to study plant sulfhydryl substances as they may be related to growth. This first paper is an examination of some non-enzymatic reactions with growth regulators. A preliminary report on part of this material has appeared elsewhere (9).

Materials and Methods

Sources of the various materials included: maleimides kindly donated by Dr. J. van Overbeek; chelidonic acid originally prepared by Dr. E. Ramstad and recrystalized from water; CoA[3] donated by the Pabst Laboratories; phosphotransacetylase donated by Dr. E. R. Stadtman through the courtesy of Dr. H. Beevers. All other reagents were obtained commercially. The TIBA was treated with charcoal and recrystallized from ethanol.

Sulfhydryl compounds were made up fresh daily and kept chilled until use. For reactions in ordinary test tubes, the sulfhydryl was added last, the tubes placed in a desiccator and evacuated. Thunberg tubes were used as a further precaution against error due to autoxidation. In the latter case the sulfhydryl was maintained in dilute acid and not exposed to other reagents until the oxygen had been expelled. With both methods the systems were alternately evacuated and filled with nitrogen three times.

Sulfhydryl estimations were obtained by the nitroprusside test (6). A typical analysis consisted of mixing 0.5 ml of sample with 5 ml saturated NaCl and 1.0 ml 2 % sodium nitroprusside, followed by 1.0 ml of a mixture of 1.5 M sodium carbonate and 0.025 M sodium cyanide. The extinction of the resulting violet color was then determined at 520 $m\mu$ on a Bausch and Lomb "Spectronic 20" or Beckman DU spectrophotometer. With the Thunberg tube technique it was possible to complete the sulfhydryl analysis within less than 1 minute after the tube was opened; with the desiccator method a batch of tubes were exposed to air while analyses were proceeding. Variations due presumably to autoxidations were consequently greater with the latter method.

CoA was measured by the phosphotransacetylase assay of Stadtman (19).

Chromatographic techniques and solvent systems used were those of Gutcho and Laufer (7). The ascending method was used. Since temperature control was not obtained, there was some variation in R_f values from one run to another.

Experimental

A survey of various growth regulators led to the finding that TIBA reacts non-enzymatically with such

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[3] Abbreviations employed are as follows: 2,4-D, 2,4,-dichlorophenoxyacetic acid; BAL, 2,3-dithiopropanol; CoA, coenzyme A; GSH, glutathione; GSSG, oxidized glutathione; TIBA, 2,3,5-triiodobenzoic acid; TRIS, tris (hydroxymethyl) aminomethane.